

A prospective study of naturally acquired sensitization and subsequent desensitization to mosquito bites and concurrent antibody responses

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We report the first prospective study of development of human sensitization and eventual desensitization after long-term natural exposure to an allergen, with concurrent immunologic responses.

Mosquito allergy, immediate and delayed reactions to the saliva injected by a mosquito bite, is caused by IgE- and lymphocyte-mediated hypersensitivities¹⁻³ and is potentially an excellent model for the study of human allergy. Most people develop local skin reactions to mosquito bites, but systemic reactions are extremely rare. Skin-immediate and delayed reactions can be measured precisely. Species-shared and species-specific antigens have been found.³ It is easy to identify a mosquito species to which a subject has not been previously exposed.

We hypothesized that controlled, regular, long-term exposure to mosquito bites from a species not previously encountered would provide a longitudinal model of sensitization and desensitization to an allergen. In a pilot study we prospectively monitored skin and antibody responses during such exposure in one human subject and one rabbit.

METHODS

Maintenance of a mosquito *Culex* (*Cx.*) *quinquefasciatus* colony, preparation of salivary gland extract, mosquito bite tests, and measurement of *Cx. quinquefasciatus* salivary gland-

specific IgE and IgG (*Cx. quinquefasciatus* IgE or IgG) by ELISA were performed as previously described.¹⁻³

After obtaining ethics committee approval and written informed consent, a 23-year-old healthy male who had never been bitten previously by *Cx. quinquefasciatus* mosquitoes received approximately 100 bites from female laboratory-reared *Cx. quinquefasciatus* mosquitoes every 2 weeks for 10 months. Two screened cages, each containing 70 to 80 mosquitoes, were placed on the volar surface of the subject's arm for 15 to 30 minutes until 70% of the mosquitoes were satiated with blood. On the opposite arm, a test bite was performed with one *Cx. quinquefasciatus* mosquito. The immediate wheal reaction to the test bite was measured 20 minutes after the bite, and the delayed reaction was measured 24 hours later. Every 4 weeks, 2 ml of serum were collected and stored at -70° C. At the end of the study, serum *Cx. quinquefasciatus* IgE and IgG levels were measured.¹

In a parallel study, a rabbit received approximately 140 bites and a bite test on the shaved back from laboratory-reared *Aedes* (*Ae.*) *aegypti* mosquitoes every week for 10 months. This study was carried out in a similar manner, except that each screened cage contained approximately 100 *Ae. aegypti* mosquitoes. The mosquito-bite protocol and the *Ae. aegypti* bite test were performed every week, serum samples were collected every 2 weeks, and only *Ae. aegypti* saliva-specific IgG levels were measured.

RESULTS

Initially, no skin reactions to the mosquito bites were noted in either the human or the rabbit. In the human the development and subsequent loss of the immediate wheal and delayed induration responses, and the rise and fall of *Cx. quinquefasciatus* IgE and IgG are shown in Fig. 1, A. *Cx. quinquefasciatus* IgE and IgG seemed to correlate with the development of skin reactivity. The development and subsequent loss of the immediate wheal and delayed induration responses and the *Ae. aegypti*-specific IgG response in the rabbit are shown in Fig. 1, B.

In the human *Cx. quinquefasciatus*-specific IgG

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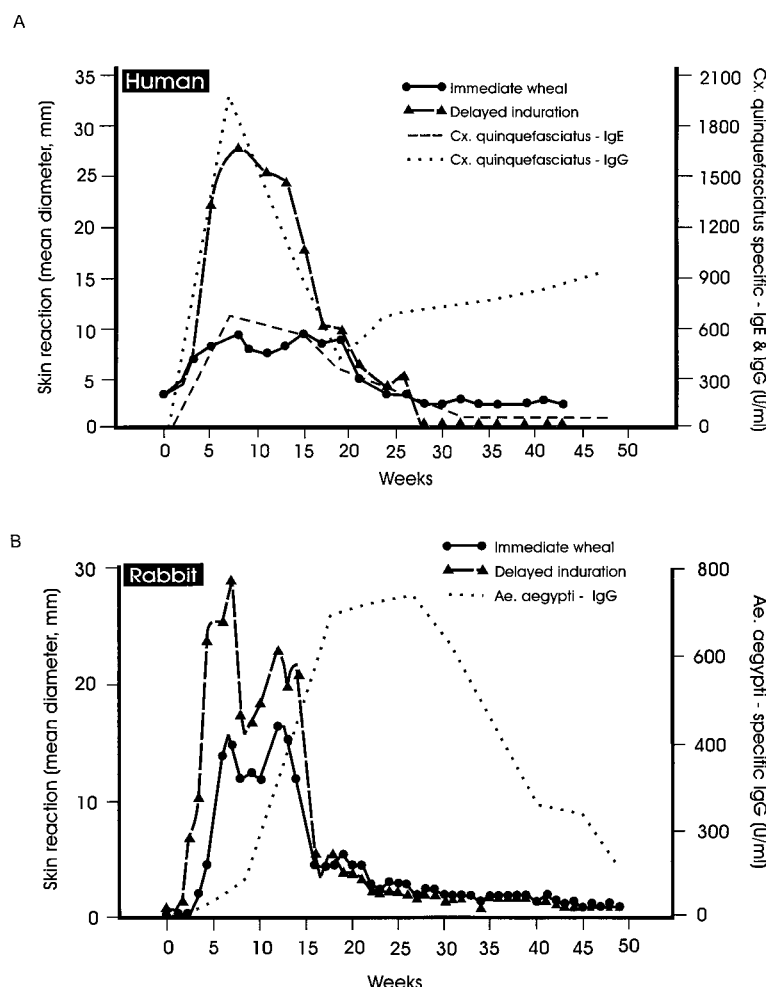


FIG. 1. Naturally acquired sensitization and subsequent desensitization to mosquito bites. **A.** In the subject, who received 100 *Cx. quinquefasciatus* bites every 2 weeks, delayed and immediate skin reactions developed at week 3 and peaked at weeks 5 to 15 and 5 to 19, respectively, and returned to baseline by week 26. *Cx. quinquefasciatus*-specific IgE and IgG peaked at week 5 concurrently with the skin reactions. The IgE level gradually decreased to baseline by week 26. The IgG level decreased to 30% of the peak by week 19 and remained low until week 39. **B.** In contrast, in a rabbit that received 140 *Ae. aegypti* bites every week, the delayed reaction developed at week 2.5 and peaked at weeks 4 to 14. The immediate reaction appeared at week 4 and peaked between weeks 6 and 14. Both skin reactions decreased by week 16 and remained small until the end of the study at week 49. The *Ae. aegypti*-specific IgG level was low when skin reactions were peaking but started to rise significantly at week 13 when the skin reactions started to decrease. The IgG response peaked during weeks 18 to 35 after the skin reactions had almost returned to baseline and then gradually fell.

seemed to correlate with the development of skin reactivity. In the rabbit *Ae. aegypti*-specific IgG seemed to develop as a blocking antibody, peaking as skin reactivity decreased.

DISCUSSION

Natural desensitization to mosquito bites develop eventually after long-term exposure, as documented in a recent cross-sectional study in which the size of the skin reactions to mosquito bites correlated inversely with the duration of exposure.²

In this study the clinical response to test mosquito bites in the human was monitored until desensitization occurred. Both the *Cx. quinquefasciatus* IgG and IgE

responses seemed to parallel the skin reactions, which was in agreement with our previous finding that specific IgG and IgE levels were significantly increased in subjects with positive bite reactions and correlated with the sizes of both the immediate and delayed reactions.² This suggests that specific IgG is involved in the development of mosquito allergy. In other cross-sectional studies of mosquito bites, IgG antibodies have been reported to be elevated⁴ and not elevated,⁵ probably because in different studies the tests were performed at different stages of the sensitization/desensitization process.

In contrast to the human, IgG in the rabbit appeared to act as a blocking antibody, which correlated with the

development of desensitization. The time course of acquired sensitization and desensitization also differed between the human and the rabbit, with the rabbit requiring a shorter time to develop desensitization (14 weeks) than the human (21 weeks). This could represent a true difference. It could also have occurred because the human had less frequent mosquito bites than the rabbit and received a relatively low dose of mosquito salivary antigen (100 bites every 2 weeks in a 70 kg human vs 140 bites every week in a 2 kg rabbit).

We demonstrate prospectively for the first time that regular, continuous, high-dose, natural allergen exposure eventually leads to desensitization (defined as complete loss of delayed and immediate responsiveness) and that both IgE and IgG are involved in the sensitization process. This model offers an excellent opportunity for further in-depth study of immediate and delayed hypersensitivity responses in humans. The immunologic mechanisms involved may be different in humans than in animals (at least in rabbits) in which IgG acts as a blocking antibody.

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REFERENCES

1. Peng Z, Yang M, Simons FER. Measurement of mosquito *Aedes vexans* salivary gland-specific IgE and IgG antibodies and the distribution of these antibodies in human sera. *Ann Allergy Asthma Immunol* 1995;74:259-64.
2. Peng Z, Yang M, Simons FER. Immunologic mechanisms in mosquito allergy: correlation of skin reactions with specific IgE and IgG antibodies and lymphocyte proliferation response to mosquito antigens. *Ann Allergy Asthma Immunol* 1996;77:238-44.
3. Li H, Simons FER, Peng Z. Immunoblot analyses of salivary antigens in 10 mosquito species [abstract]. *J Allergy Clin Immunol* 1996;97:337.
4. Ailus K, Palosuo T, Brummer-Korvenkontio H, Rantanen T, Reunala T. Demonstration of antibodies to mosquito antigens in man by immunodiffusion and ELISA. *Int Arch Allergy App Immunol* 1985;78:375-9.
5. Penneys NS, Nayar JK, Bernstein H, Knight JW. Circulating antibody detection in human serum to mosquito salivary gland proteins by the avidin-biotin-peroxidase technique. *J Am Acad Dermatol* 1988;18:87-92.